TPLGPASSLPQSFLLKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIPWA
PLSSCPSQALQLAGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQLDVADFATTIW
QQMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRHLAQP

hGCSF wild type

ATGACTCCATTAGGTCCAGCTTCCTCTCTGCCGCAAAGCTTCCTGCTGAAATGCCTGGAACAGGTTCGTAAAATCCAGGGTGATGG
TGCTGCTCTGCAGGAAAAACTGTGCGCTACCTACAAACTGTGCCATCCGGAAGAACTGGTTCTGCTGGGTCACTCCCTGGGTATCC
CGTGGGCGCCGCTGAGCTCCTGCCCGAGCCAGGCTCTGCAGCTGGCTGGTTGCCTGTCCCAATTGCACAGCGGCCTTTTCCTGTAC
CAGGGTCTGCTGCAAGCTCTGGAAGGTATCTCCCCGGAACTGGGTCCGACCCTGGACACTCTGCAGCTGGACGTCGCTGACTTCGC
TACCACCATCTGGCAGCAGATGGAAGAACTGGGTATGGCTCCGGCTCTGCAGCCGACCCAGGGTGCTATGCCGGCTTTCGCTTCCG
CTTTCCAGCGTCGCGCAGGTGGCGTTCTGGTTGCTAGCCACCTGCAGAGCTTCCTGGAAGTTTCCTACCGTGTTCTGCGTCACCTG
GCTCAGCCGTGA

FIG._1

core3	17	21	24	28	31	35	41	47	54	56	75	78	82	85	88	89	92
	95	99	103	106	110	113	114	117	140	151	152	153	154	157	160	161	168
core4	17	21	24	28	31	75	78	82	85	89	103	106	110	113	114	117	149
	150	151	152	153	154	157	160	161	168								
core4v	17	21	24	28	31	75	78	82	85	89	103	106	110	113	114	117	151
	152	153	154	157	160	161	167	168									
bndry4_2		14	20	27	32	34	38	77	79	84	91	99	102	107	109	116	
		120	145	146	147	148	155	156	164	170							
bndry4_core4		14	20	27	32	34	38	77	79	84	91	99	102	107	109	116	
		120	145	146	147	148	155	156	164	170							
bndry4_AD		14	20	27	32	34	38	145	146	147	148	155	156	164	170		
bndry4_AD_core4		14	20	27	32	34	38	145	146	147	148	155	156	164	170		

FIG._2

G-CSF Designs - Optimal Sequences Selected by PDA*

```
                1         10        20        30        40        50        60
hGCSFwt    MTPLGPASSLPQSFLLKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIPWAP
bndry4_2                  I     L       E    I I   K
bndry4_core4              I  L  L       EA   L E   H
bndry4_AD                 I     L       E    I E   H
bndry4_AD_core4           I  L  L       EA   L E   H
core4                        L             A
core4_V167A                  L   I         A
core3                        L             A        I
-------------------------------------------------------------------------------
sm0                          A             A
fm2                          A             A
fm3                          L             A
fm4                          L             A
fm7                          L             A

                   70        80        90        100       110       120
hGCSFwt    LSSCPSQALQLAGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQLDVADFATTIWQQ
bndry4_2                    L           K       V  I    I E       I   L
bndry4_core4                FL     F    K          KV   I EI  L  I   L
bndry4_AD
bndry4_AD_core4             F      F                  V       I  L
core4                       F      F                  V       I  L
core4_V167A                 F      F                          I  L
core3                       F      F   F  F                      L
-------------------------------------------------------------------------------
sm0
fm2
fm3                         F                                     L
fm4                         F                                     L
fm7                         F      F                              L
```

FIG._3A

AA-9/9147-2

	130	140	150	160	170
hGCSFwt	MEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRHLAQP				
bndry4_2			KED IL A		
bndry4_core4			KED **I I** IL A **F**		
bndry4_AD			KET IL A		
bndry4_AD_core4			KED **I I** IL A **F**		
core4			**I I** **F**		
core4_V167A			**I I** I WF **AF**		
core3			**I I** **F**		
sm0					
fm2			**I I**		
fm3			**F**		
fm4			**I I** **F**		
fm7			**I I** **F**		

*Sequences shown below dotted lines were not obtained from PDA calculations but were derived by reverting some core4 or core3 mutant positions to wild type. Core4 mutant positions are indicated in bold. The sequence selected for Core4_V167A is not the ground state; Monte Carlo analysis shows the ground state with Phe instead of Trp for position 160, and Leu instead of Phe for position 161 (see Table 4).

FIG._3B

Core4 - Monte Carlo Analysis - Ground State and Allowed Amino Acids and Their Number of Occurrences (For theTop 1000 Sequences)

hG-CSF	Position	Ground State					
CYS	17	LEU	736	ILE	229		
VAL	21	VAL	687	ILE	287		
ILE	24	VAL	38	ILE	961		
GLY	28	ALA	747	LEU	172		
LEU	31	VAL	251	LEU	707		
LEU	75*	LEU	999				
LEU	78	PHE	974				
LEU	82*	LEU	974				
TYR	85	PHE	847	TYR	140		
LEU	89	LEU	628	PHE	321		
LEU	103	VAL	351	LEU	264	ILE	313
LEU	106*	LEU	940				
VAL	110	VAL	415	LEU	143	ILE	441
PHE	113	LEU	999				
ALA	114*	ALA	999				
ILE	117*	ILE	956				
GLY	149*	GLY	999				
GLY	150*	GLY	999				
VAL	151	ILE	999				
LEU	152*	LEU	999				
VAL	153	VAL	411	ILE	588		
ALA	154*	ALA	999				
LEU	157	LEU	805	ILE	187		
PHE	160	PHE	565	TRP	434		
LEU	161	LEU	838	PHE	161		
LEU	168	PHE	999				

*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type

FIG._4

Table 4. Core4v - Monte Carlo Analysis (Ground State and Allowed Amino Acids and Their Number of Occurrences (For the Top 1000 Sequences)

hG-CSF	Position	Ground State							
CYS	17	LEU	697	VAL	51	ILE	251		
VAL	21	VAL	682	VAL	682	ILE	300	PHE	17
ILE	24	ILE	938	VAL	61				
GLY	28	ALA	806	LEU	193				
LEU	31	LEU	694	ALA	1	VAL	257	ILE	47
LEU	75*	LEU	999						
LEU	78	PHE	982	VAL	17				
LEU	82	LEU	982	PHE	17				
TYR	85	PHE	887	VAL	2	ILE	16	TYR	94
LEU	89	LEU	637	PHE	314	TRP	48		
LEU	103	VAL	357	ALA	78	LEU	269	ILE	295
LEU	106	LEU	945	VAL	54				
VAL	110	ILE	445	VAL	405	LEU	149		
PHE	113	LEU	999						
ALA	114*	ALA	999						
ILE	117*	ILE	938	VAL	61				
VAL	151	ILE	999						
LEU	152*	LEU	999						
VAL	153	ILE	585	VAL	414				
ALA	154*	ALA	999						
LEU	157	LEU	797	VAL	18	ILE	184		
PHE	160	PHE	551	TRP	448				
LEU	161	LEU	843	PHE	156				
VAL	167	ALA	999						
LEU	168	PHE	999						

*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type

FIG._5

Table 5. Core3 - Monte Carlo Analysis (Ground State and Allowed Amino Acids and Their Number of Occurrences (For the Top 1000 Sequences)

hG-CSF	Position	Ground State											
CYS	17	LEU	585	VAL	35	ILE	379						
VAL	21	VAL	551	ALA	15	ILE	291	PHE	141	TYR	1		
ILE	24	ILE	657	ALA	31	VAL	303	LEU	8				
GLY	28	ALA	928	LEU	71								
LEU	31	LEU	888	VAL	111								
LYS	35	ILE	785	VAL	214								
LEU	41*	LEU	999										
LEU	47*	LEU	999										
LEU	54*	LEU	999										
ILE	56*	ILE	999										
LEU	75*	LEU	999										
LEU	78	PHE	692	ALA	10	VAL	149	LEU	12	ILE	75	TYR	61
LEU	82	LEU	851	ALA	12	PHE	136						
TYR	85	PHE	636	TRP	363								
LEU	88*	LEU	999										
LEU	89	PHE	674	LEU	214	TRP	111						
LEU	92	PHE	999										
ILE	95*	ILE	999										
LEU	99*	LEU	999										
LEU	103	LEU	888	ILE	111								
LEU	106	LEU	893	VAL	106								
VAL	110	VAL	400	ALA	14	LEU	294	ILE	291				
PHE	113	LEU	954	ALA	1	PHE	44						
ALA	114*	ALA	999										
ILE	117	ILE	790	ALA	15	VAL	168	LEU	5	PHE	20	TRP	1

FIG._6A

Table 5. Core3 - Monte Carlo Analysis (Ground State and Allowed Amino Acids and Their Number of Occurrences (For the Top 1000 Sequences)

hG-CSF	Position	Ground State								
PHE	140*	PHE	999							
VAL	151	ILE	999							
LEU	152*	LEU	999							
VAL	153	ILE	999							
ALA	154*	ALA	999							
LEU	157	LEU	694	ALA	22	VAL	179	ILE	104	
PHE	160	PHE	574	TRP	425					
LEU	161	LEU	784	ALA	6	VAL	55	PHE	154	
LEU	168	PHE	999							

*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type

FIG._6B

Table 6. Bndry4_2 - Monte Carlo Analysis (Ground State and Allowed Amino Acids and Their Number of Occurrences (For the Top 1000 Sequences)

hG-CSF	Position	Ground State								
LEU	14	ILE	998							
GLN	20	LEU	999							
ASP	27	GLU	999							
GLN	32	ILE	999							
LYS	34	LYS	717	ILE	209	GLU	73			
THR	38	VAL	409	ILE	188	GLU	237	LYS	154	
GLN	77*	GLN	999							
HIS	79	LEU	999							
LEU	84*	LEU	999							
ALA	91	LYS	999							
LEU	99	VAL	759	LEU	193					
THR	102	LEU	562	ILE	404					
GLN	107	ILE	993							
VAL	109	GLU	525	VAL	474					
THR	116	ILE	749	LEU	198	LYS	52			
GLN	120	LEU	999							
GLN	145	GLN	650	GLU	349					
ARG	146	LYS	891	GLN	108					
ARG	147	GLU	999							
ALA	148	THR	401	ALA	268	ASP	330			
SER	155	ILE	999							
HIS	156	LEU	999							
SER	164	ALA	999							
HIS	170	HSP	380	LEU	111	GLU	248	GLN	227	

*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type

FIG._7

Table 7. Bndry4_core4 - Monte Carlo Analysis (Ground State and Allowed Amino Acids and Their Number of Occurrences (For the Top 1000 Sequences)

hG-CSF	Position	Ground State											
LEU	14	ILE	941	LEU	58	ILE	941						
GLN	20	LEU	999										
ASP	27	GLU	970	SER	29								
GLN	32	LEU	631	VAL	125			ILE	243				
LYS	34	GLU	961	GLN	22	LYS	16						
THR	38	HSP	931	VAL	19	ILE	4	GLU	5	LYS	40		
GLN	77*	GLN	999										
HIS	79	LEU	999										
LEU	84*	LEU	999										
ALA	91	LYS	999										
LEU	99	LEU	922	GLU	77								
THR	102	LYS	729	THR	14	VAL	150	LEU	2	ILE	71	GLU	14	GLN	19
GLN	107	ILE	968	VAL	30	LEU	1						
VAL	109	GLU	591	VAL	402	ASP	2	GLN	4				
THR	116	ILE	647	VAL	15	LEU	275	GLU	1	LYS	61		
GLN	120	LEU	999										
GLN	145	GLN	658	GLU	341								
ARG	146	LYS	857	GLN	142								
ARG	147	GLU	998	LYS	1								
ALA	148	ASP	359	ALA	310	THR	330						
SER	155	ILE	999										
HIS	156	LEU	999										
SER	164	ALA	999										
HIS	170	HSP	380	ASP	26	LEU	109	GLU	239	GLN	214	LYS	31

*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type

FIG._8

Table 8. Bndry4_AD - Monte Carlo Analysis (Ground State and Allowed Amino Acids and Their Number of Occurrences (For the Top 1000 Sequences)

Position	Ground State											
14	ILE	887	LEU	112								
20	LEU	999										
27	GLU	984										
32	ILE	931										
34	GLU	357	ILE	68	GLN	223	LYS	277				
38	VAL	287	ILE	133	HSP	225	GLU	217	LYS	123		
145	GLN	605	GLU	394								
146	LYS	786	GLN	213								
147	GLU	962										
148	THR	373	ALA	305	ASP	321						
155	ILE	976										
156	LEU	994										
164	ALA	999										
170	HSP	304	ASP	55	LEU	136	GLU	230	GLN	209	LYS	62

*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type

FIG._9

Table 9. Bndry4_AD_core4 - Monte Carlo Analysis (Ground State and Allowed Amino Acids and Their Number of Occurrences (For the Top 1000 Sequences)

Position	Ground State									
14	ILE	896	LEU	103						
20	LEU	999								
27	GLU	996								
32	LEU	523	VAL	194	ILE	271				
34	GLU	400	GLN	207	LYS	341				
38	VAL	300	ILE	89	HSP	277	GLU	203	LYS	130
145	GLN	623	GLU	376						
146	LYS	820	GLN	179						
147	GLU	986								
148	ASP	344	ALA	332	THR	323				
155	ILE	998								
156	LEU	996								
164	ALA	999								
170	HSP	330	LEU	134	GLU	234	GLN	216		

*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type

FIG._10

Core3

ATGACTCCATTAGGTCCAGCTTCCTCTCTGCCGCAAAGCTTCCTGCTGAAACTGCTGGAACAGGTTCGTAAAATCCAGGGTGATGC
AGCTGCTCTGCAGGAAAAAATCTGCGCTACCTACAAACTGTGCCATCCGGAAGAACTGGTTCTGCTGGGTCACTCCCTGGGTATCC
CGTGGGCGCCGCTGAGCTCCTGCCCGAGCCAGGCTCTGCAGCTGGCTGGTTGCCTGTCCCAATTCCACAGCGGCCTTTTCCTGTTC
CAGGGTCTGTTCCAGGCTTTCGAAGGTATCTCCCCGGAACTGGGTCCGACCCTGGACACTCTGCAGCTGGACGTCGCTGACCTGGC
TACCACCATCTGGCAGCAGATGGAAGAACTGGGTATGGCTCCGGCTCTGCAGCCGACCCAGGGTGCTATGCCGGCTTTCGCTTCCG
CTTTCCAGCGTCGCGCAGGTGGCATCCTGATCGCTAGCCACCTGCAGAGCTTCCTGGAAGTTTCCTACCGTGTTTTCCGTCACCTG
GCTCAGCCGTGA

FIG._11A

Core4

ATGACTCCATTAGGTCCAGCTTCCTCTCTGCCGCAAAGCTTCCTGCTGAAACTGCTGGAACAGGTTCGTAAAATCCAGGGTGATGC
AGCTGCTCTGCAGGAAAAACTGTGCGCTACCTACAAACTGTGCCATCCGGAAGAACTGGTTCTGCTGGGTCACTCCCTGGGTATCC
CGTGGGCGCCGCTGAGCTCCTGCCCGAGCCAGGCTCTGCAGCTGGCTGGTTGCCTGTCCCAATTCCACAGCGGCCTTTTCCTGTTC
CAGGGTCTGCTGCAAGCTCTGGAAGGTATCTCCCCGGAACTGGGTCCGACCGTTGACACTCTGCAGCTGGACATCGCTGACCTGGC
TACCACCATCTGGCAGCAGATGGAAGAACTGGGTATGGCTCCGGCTCTGCAGCCGACCCAGGGTGCTATGCCGGCTTTCGCTTCCG
CTTTCCAGCGTCGCGCAGGTGGCATCCTGATCGCTAGCCACCTGCAGAGCTTCCTGGAAGTTTCCTACCGTGTTTTCCGTCACCTG
GCTCAGCCGTGA

FIG._11B

Core4v

ATGACTCCATTAGGTCCAGCTTCCTCTCTGCCGCAAAGCTTCCTGCTGAAACTGCTGGAACAGATCCGTAAAATCCAGGGTGATGC
AGCTGCTCTGCAGGAAAAACTGTGCGCTACCTACAAACTGTGCCATCCGGAAGAACTGGTTCTGCTGGGTCACTCCCTGGGTATCC
CGTGGGCGCCGCTGAGCTCCTGCCCGAGCCAGGCTCTGCAGCTGGCTGGTTGCCTGTCCCAATTCCACAGCGGCCTTTTCCTGTTC
CAGGGTCTGCTGCAAGCTCTGGAAGGTATCTCCCCGGAACTGGGTCCGACCCTGGACACTCTGCAGCTGGACATCGCTGACCTGGC
TACCACCATCTGGCAGCAGATGGAAGAACTGGGTATGGCTCCGGCTCTGCAGCCGACCCAGGGTGCTATGCCGGCTTTCGCTTCCG
CTTTCCAGCGTCGCGCAGGTGGCATCCTGATCGCTAGCCACATCCAGAGCTGGTTCGAAGTTTCCTACCGTGCTTTCCGTCACCTG
GCTCAGCCGTGA

FIG._11C

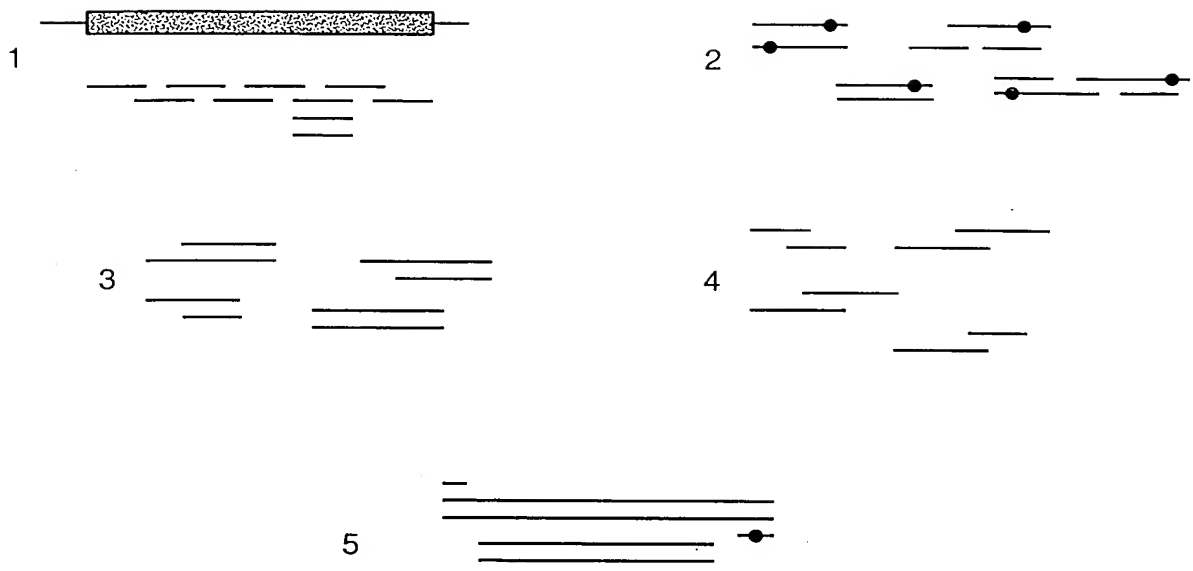

FIG._12

-Melting Temperature (T_m)

	T_m (°C)	Extinction Coefficient (M^{-1} cm^{-1})
hG-CSFwt	60	15720
core4	72	14230
core4v	61	19730
core3	58	14230
sm0*	63	15720
fm4*	63	15720
fm7*	70	14230

* Derived by reverting some core4 or core3 mutant positions to wild type

FIG._16

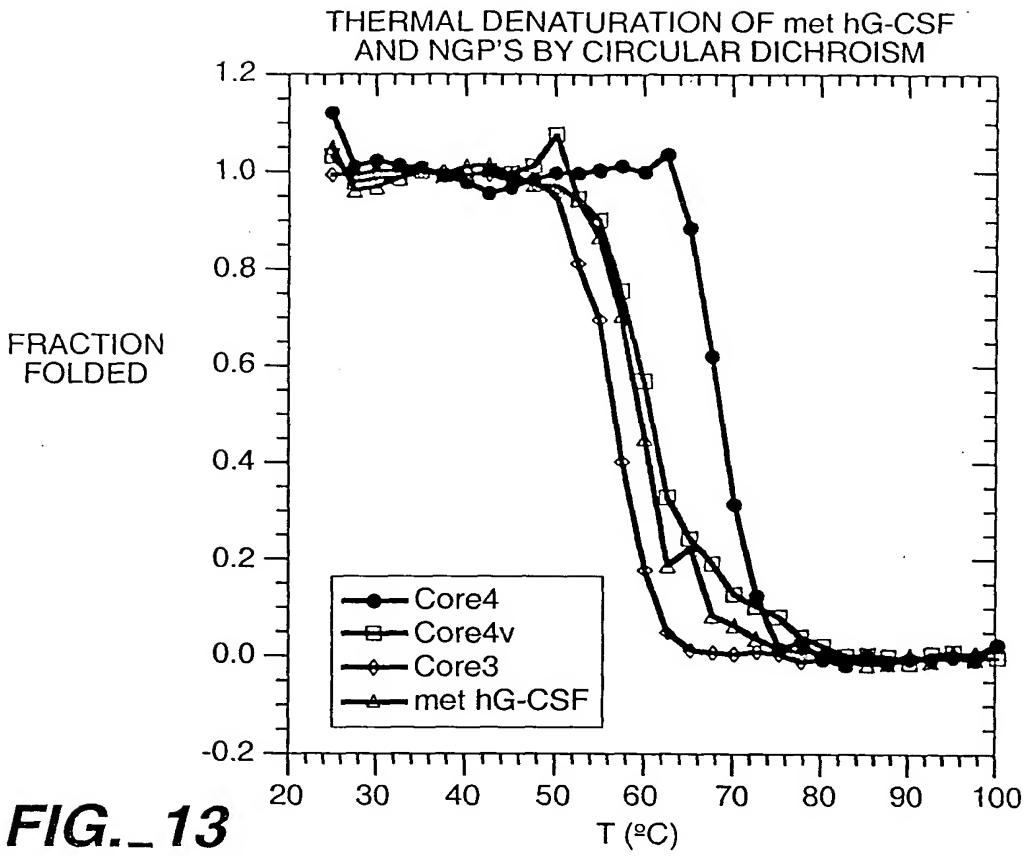
THERMAL DENATURATION OF met hG-CSF
AND NGP'S BY CIRCULAR DICHROISM
FRACTION
FOLDED
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
20
30
40
50
60
70
80
90
100
T (ºC)
Core4
Core4v
Core3
met hG-CSF

FIG._13

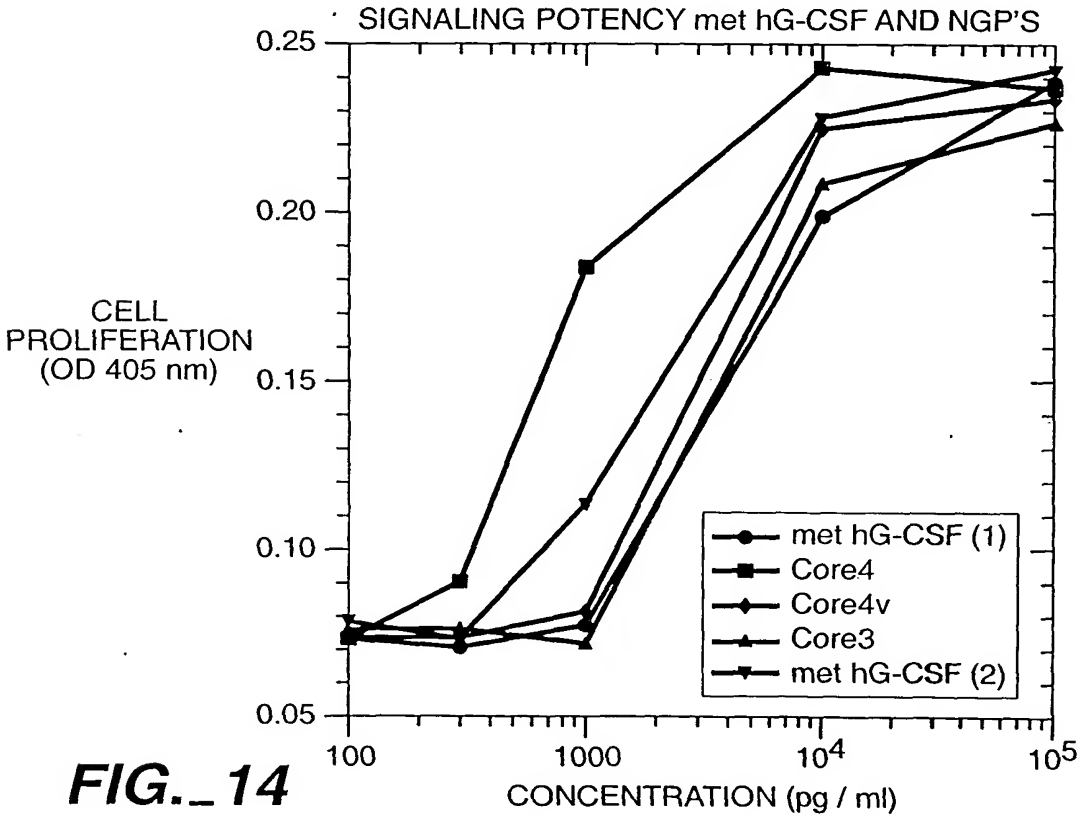
SIGNALING POTENCY met hG-CSF AND NGP'S
CELL
PROLIFERATION
(OD 405 nm)
0.25
0.20
0.15
0.10
0.05
100
1000
10^4
10^5
CONCENTRATION (pg / ml)
met hG-CSF (1)
Core4
Core4v
Core3
met hG-CSF (2)

FIG._14

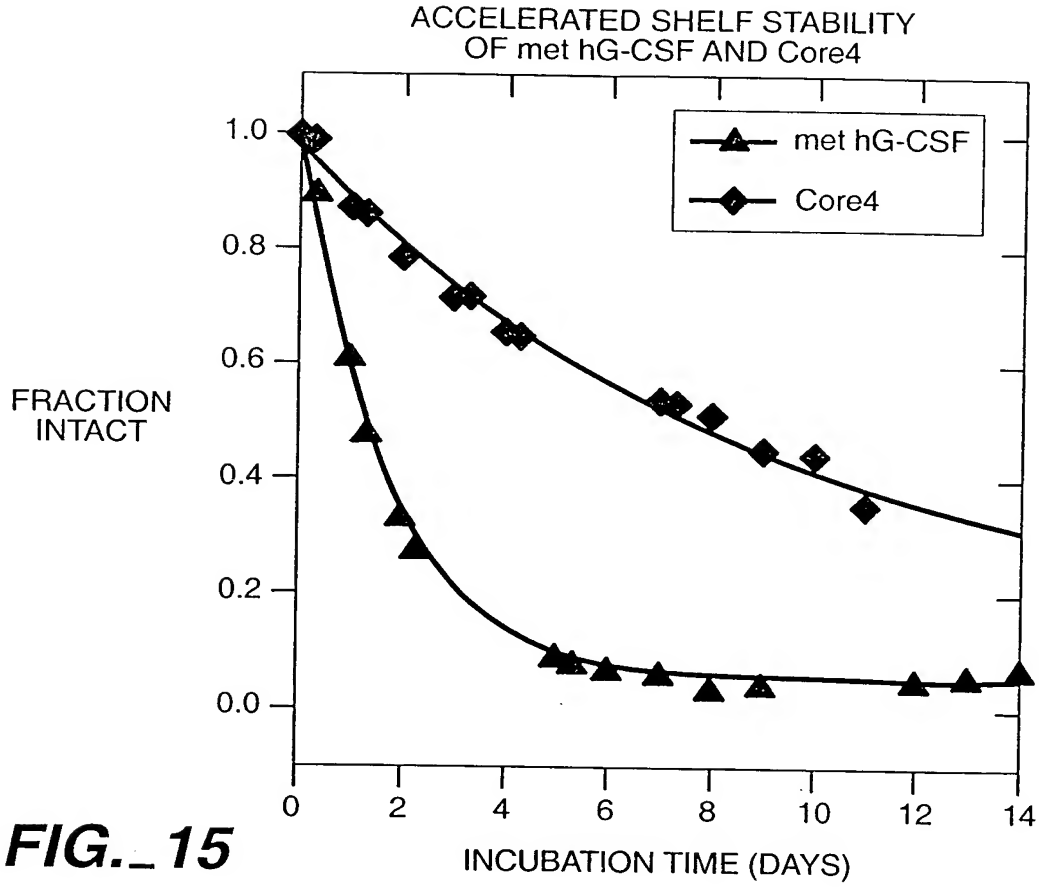
ACCELERATED SHELF STABILITY
OF met hG-CSF AND Core4
met hG-CSF
Core4
FRACTION
INTACT
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
INCUBATION TIME (DAYS)

FIG._15